EXHIBIT 1

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Clinical Report

Small Familial Supernumerary Ring Chromosome 2: FISH Characterization and Genotype-Phenotype Correlation

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A child and his mother were found to be mosaic for a small supernumerary marker chromosome (SMC) that was identified and characterized by means of fluorescent in situ hybridization. The marker chromosome was derived from the pericentromeric region of chromosome 2; the involvement of proximal 2q was determined by YAC probes. The proband was referred because of psychotic illness and mild mental retardation, whereas his mother presented only minor dysmorphisms. There are only a few published reports concerning SMC(2) or proximal 2q trisomy. We reviewed the previously reported cases in an attempt to establish genotype-phenotype correlations, which are particularly important when SMCs are identified in prenatal diagnosis.

KEY WORDS: supernumerary marker chromosome; chromosome 2q partial trisomy; clinical phenotype

INTRODUCTION

Supernumerary marker chromosomes (SMCs) derived from all autosomes have been identified and reviewed on the basis of their chromosomal origin [Crolla et al., 1998; Stankiewicz et al., 2000], but the clinical consequences of those containing pericentromeric regions remain unclear. Supernumerary ring chromosomes (SRCs) account for about 10% of SMCs [Blennow et al., 1994].

To the best of our knowledge, there are only four published reports concerning SRCs(2); all cases were mosaics: two had phenotypical abnormalities [Plattner et al., 1993; Ostroverkhova et al., 1999] while the others are phenotypically normal [Daniel et al., 1994; Villa et al., 2001]. The differences in the clinical symptoms of patient with SMCs of the same chromosomal origin are probably due to variations in genetic content, the degree of mosaicism, and the possible presence of uniparental disomy [Rothlisberger et al., 2000].

We here describe the fluorescent in situ hybridization (FISH) characterization of SRCs(2) observed in a mother with minor dysmorphic features and her child, who was affected by mild mental retardation, facial dysmorphisms, and psychotic illness. Because of the presence of a clinical phenotype, the SRCs were further investigated using pericentromeric YAC probes with the aim of detecting imbalance of defined chromosome 2 pericentromeric loci.

CLINICAL REPORTS

Patient 1

The 6-year-old male proband is the first child of a 35-year-old woman and a 37-year-old man. The parents are healthy and nonconsanguineous. He was delivered at 40 weeks without any complications. His birth weight and length were, respectively, 3.020 kg (25th centile) and 49 cm (25th centile), and both his height and weight had remained in the normal range since early infancy. He was referred for cytogenetic analysis because of psychotic illness and mild mental retardation.

At the time of clinical evaluation, height was 120 cm (90th centile), weight 19 kg (25th centile), and

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occipitofrontal head circumference (OFC) 49 cm (below the 3rd centile). He presented with mild brachycephaly, a long face, deep-set eyes, and a prominent nasal columella. A neuropsychological examination revealed mild mental retardation, psychotic behavior with attention deficit, hyperactivity, and stereotypic movements.

Patient 2

The mother was examined following the finding in her karyotype of an SMC similar to the marker discovered in her child's karyotype. Her neonatal and childhood history were unremarkable, characterized by normal growth and neuropsychological development. After her first pregnancy (which led to the proband's birth), three further pregnancies were terminated because of spontaneous abortion occurring during the early weeks of gestation. A clinical examination revealed only minor dysmorphisms consisting of a small mandible, low-set ears, downslanting palpebral fissures, and a prominent nasal columella.

MATERIALS AND METHODS

Peripheral blood lymphocyte cultures and chromosome spreads were set up according to standard methods. The karyotype analysis (according to the ISCN [1995]) was performed on QFQ-stained metaphases. FISH by means of chromosome 2 painting library (WCP2), D2Z probe, and All Human Telomeres Probe (Oncor) followed the protocol of the supplier. YAC clones encompassing chromosome 2 pericentromeric sequences belonging to WC2.7, WC2.8, and WC2.9 contigs (http:/www.genome.wi.mit.edu) were provided by the YAC Screening Center (DIBIT, Milan, Italy). All probes were labeled by nick-translation with biotin or digoxigenin (Roche, Switzerland). The protocols for YAC FISH were according to Lichter and Cremer [1992].

Mat 2 uniparental disomy (UPD) segregation analysis from parents to proband was performed on lymphocyte DNAs by using six chromosome 2 microsatellites (D2S305, D2S391, D2S286, D2S347, D2S364, and D2S206).

RESULTS

Cytogenetic analysis was performed on peripheral blood lymphocytes from the patient and his parents. The patient's karyotype was 47,XY,+mar/46,XY with the SMC present in 80 of 100 consecutively scored metaphases (Fig. 1a). The father's karyotype was normal,

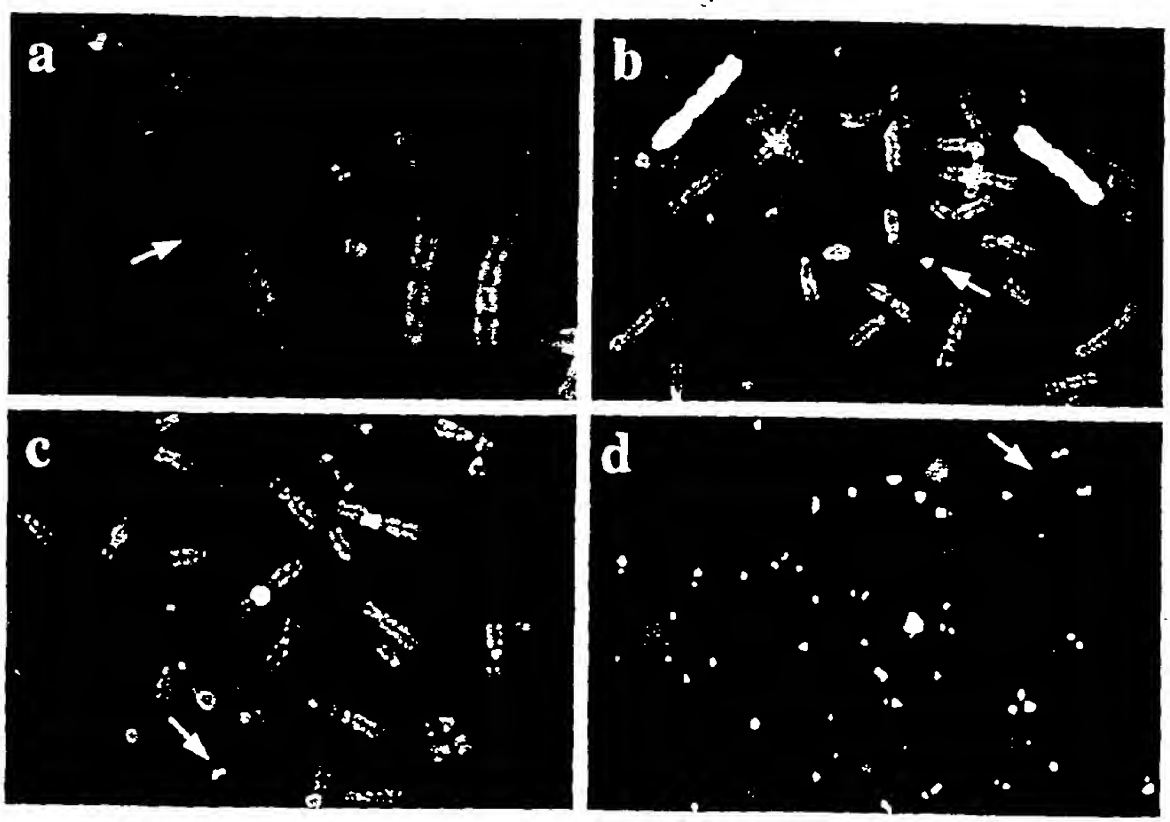

Fig. 1. Partial QFQ-banded metaphase from the proband showing the SMC: its small size can be appreciated by comparing it with that of the adjacent chromosome 21 homologues (a). FISH with WCP2 and D2Z showing that the SMC originates from chromosome 2 (b) and has a chromosome 2–specific centromeric constriction (c). FISH with All Human Telomeres Probe did not show any signal on the SMC (d), thus suggesting its ring structure. Arrows point to the marker chromosome

TABLE I. FISH Results Obtained on the SRC(2) Using WC2.7, WC2.8, and WC2.9 YAC Contigs

Probe	Contig	Markers	Localization	FISH signal on r(2)	
				Proband	Mother
747C10	WC2.7	CHLC.GATA65A06	2p11.2	–	–
914H7	WC2.7	WI-6863-NIB688-D2S2181-WI-1742-D2S2216-WI-8268	2p11.2	–	–
779F12	WC2.8	D2S113-WI-10308-WI-6746	2q11.2	+	+
972H12	WC2.8	WI-10308-WI-6746	2q11.2	+	+
964B9	WC2.8	D2S2175-WI-8436-D2S2187-D2S2311-WI-8825-D2S1309-WI1887	2q11.2	+	+
809C8	WC2.8	D2S2356-WI-10003	2q11.2	+	+
848E10	WC2.9	CHLC.GATA88C05-WI-4290-AFMB059WC1-CHLC.GATA13H01	2q12	+	+
856A4	WC2.9	CHLC.GATA13H01-WI-9518-CHLC.ATA1B01-CHLC.ATA19E11	2q12	+	+
636B10	WC2.9	D2S135	2q12	+	+
739E6	WC2.9	WI-9900-D2S1897-D2S176-WI-6081-WI-8550	2q12	–	–
957D2	WC2.9	WI-4521-D2S1897-D2S176-WI-6081-WI-8550-CHLC.GATA14H02-CHLC.GATA5G02-D2S293-D2S1894-CHLC.GATA68D08	2q12	–	–

but the mother's karyotype was 47,XX,+mar/46,XX, with the SMC identified in 54% of metaphases. The cytogenetic analysis was extended to the maternal grandparents, who had normal karyotypes.

In order to define the origin of the SMC, chromosome-specific probes were used for FISH analysis. The marker was positive with WCP2 and D2Z probes (Figs. 1b anc c) and could be classified as an SRC based on the lack of any signal after FISH with the All Human Telomeres Probe (Fig. 1d). The ring appeared identical by size and structure in all cells analyzed. FISH by using the same probes confirmed the chromosome 2 origin of the SMC present in the proband's mother.

FISH characterization of chromosome 2–derived pericentromeric sequences using YAC clones belonging to the WC2.7, WC2.8, and WC2.9 contigs relied on at least 10 rings of the proband and 10 of his mother. Table I lists the YACs in cen-tel order, their anchored markers and chromosomal location, and the FISH results on proband and mother SRCs(2). All of the YAC clones belonging to the WC2.8 contig showed hybridization signals on the SRC(2) (data not shown). Of the five YACs spanning the 2q12 band, y848E10, y856A4, and y636B10 (Fig. 2a) showed signals on SRC(2), whereas y739E6 (Fig. 2b) and y957D2 did not.

The 2q breakpoint could therefore be located between y636B10 mapping to the SRC(2) and y739E6, which was not present. The SRC in the patient and his mother could be defined as r(2)(q10q12), thus making them carriers of the same mosaic 2q11-q12 trisomy. The clinical phenotype of the proband (unlike the borderline phenotype of his mother) is not due to the concomitance of mat UPD(2), as biparental inheritance was observed at four informative loci (data not shown). UPD studies could not be performed on the mother because her parents did not authorize the test.

DISCUSSION

An abnormal phenotype has been observed in about 60% of the cases with SRC [Blennow and Tillberg, 1996]. Important factors, determining whether or not an SRC will give rise to a clinical phenotype, are the chromosomal material involved and the degree of

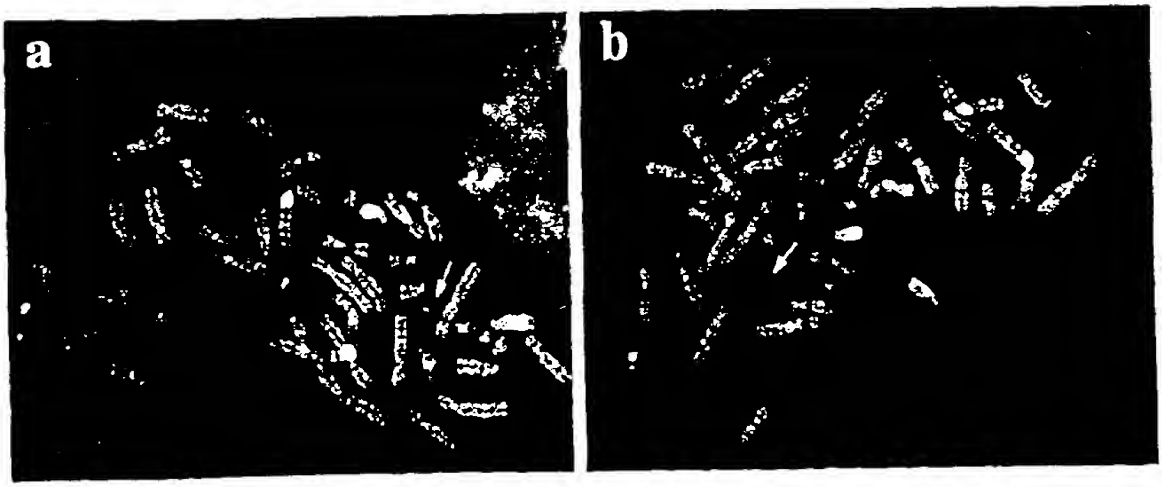

Fig 2. FISH analysis of y636B10 located at 2q12 gives a clear signal on the SRC (a), whereas the distal y739E6 does not show any signal (b). Arrows point to the marker chromosome.

TABLE II. Chromosome 2 Partial Trisomy Cases and Associated Phenotypic Finding

Studies	Tris 2	Mosaic	Clinical findings
Plattner et al. [1993]	r(2)[a]	30%	7.5 years: mental retardation, autistic behavior, hyperactivity
Daniel et al. [1994]	r(2)[a]		30 years: normal
Ostroverkhova et al. [1999]	r(2) (p11.2q14.1)	50%	18 months: developmental delay and physical anomalies; 11.5 years: mental retardation, dysmorphic features, muscular hypotonia, hypogenitalism
Mu et al. [1984]	2q11.2-q14.2		9.5 years: mental retardation, short stature, dysmorphic features, brachycephaly, prominent columella, glaucoma
Glass et al. [1998]			
Proband	2q11.2-q21.1		37 years: mild mental retardation, short stature, dysmorphic features, brachycephaly, prominent columella, major psychosis; 66 years: same phenotype of the daughter
Mother	2q11.2-q21.1		
This report			
Proband	r(2) (q10q11.2)	80%	6 years: mental retardation, psychotic behavior, hyperactivity, brachycephaly prominent columella minor dysmorphisms;
Mother	r(2) (q10q11.2)	50%	41 year: minor dysmorphisms
Villa et al. [2001][b]	r(2) (p10q11.2)	44%	16 months: normal

[a]Very small ring identified by FISH with D2Z.
[b]Ascertained during prenatal diagnosis.

mosaicism. In theory, another mechanism to be considered in relation to the clinical manifestations observed in SRC carriers is UPD, which may occur following trisomy rescue [Engel, 1993].

As chromosome 2 UPD can be excluded in our proband, his symptoms are due to partial trisomy of proximal 2q. SRCs derived from chromosome 2 are rare, to the best of our knowledge, only four cases of a very small chromosome 2–derived SRC identified by FISH have been reported [Plattner et al., 1993; Daniel et al., 1994; Ostroverkhova et al., 1999; Villa et al., 2001]. The associated phenotypes are summarized in Table II.

The clinical characteristics showed by the case described by Plattner et al. [1993] are very similar to those of our proband, but are not present in his mother carrying the same SMC(2). The symptoms of the Ostroverkhova et al. [1999] and Mu et al. [1984] patients (Table II) are similar and more severe than those observed in our patient, possibly because of the larger trisomic region. The signs observed in these two patients but not in our cases may therefore be associated with the 2q12-14.2 duplication. Two familial cases with 2q11.2-21.1 trisomy and major psychosis were reported by Glass et al. [1998].

Three of the above cases [Plattner et al., 1993; Glass et al., 1998] and our proband (i.e., four out of seven evaluable patients with duplicated proximal 2q euchromatic sequences) show psychotic/autistic behavior. One possibility is that a predisposing/causative gene maps within 2q11-12, the minimal overlapping trisomic region shared by all of the psychotic patients.

It is difficult to explain how a structurally identical SMC can underlie both a borderline and an overt clinical phenotype. UPD2, which has been shown to underlie a clinical phenotype [Stratakis et al., 2001], has been excluded in our proband, thus leaving as putative explanations the smaller percentage of cells with the SMC in the mother and the unexplored mosaicism in other uninvestigated tissues.

FISH analyses using unique sequences are useful means of obtaining information about the euchromatic regions contained in an SMC and delineating new chromosomal syndromes, aimed to offer suitable genetic counseling, especially when an SMC is observed in prenatal diagnosis.

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